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09/755,251

Set	Items	Description
S1	449	AU='ABRIGNANI S' OR AU='ABRIGNANI S.' OR AU='ABRIGNANI SERGIO' OR AU='ABRIGNANI, S.' OR AU='ABRIGNANI, SERGIO' OR AU='ABRIGNNAI SERGIO' OR AU='ABRIGO'
S2	101687	HEPATITIS(W)C(W)VIRUS
S3	79203	HCV
S4	502	NONA(W)NONB
S5	120626	S2 OR S3 OR S4
S6	227	S1 AND S5
S7	4178130	RECEPTOR? ?
S8	199	24KD
S9	3329	25(W)KD
S10	1463	CD81
S11	130	CD(W)81
S12	4181132	S7 OR S8 OR S9 OR S10 OR S11
S13	5103	S5 AND S12
S14	830	S13 NOT PY>1995
S15	678	RD (unique items)
S16	3520	S8 OR S9
S17	3	S15 AND S16
S18	1887	24(W)KD
S19	2050	S8 OR S18
S20	4182375	S12 OR S19
S21	5105	S5 AND S20
S22	111	S1 AND S21
S23	7	S22 NOT PY>1995
S24	4	RD (unique items)
S25	830	S21 NOT PY>1995
S26	678	RD (unique items)
S27	69395	S5/TI
S28	186	S26 AND S27
S29	9874	MOLT(W)4
S30	283893	SOLUBILIZ?
S31	567896	(CELL OR CELLULAR)(W)MEMBRANE? ?
S32	1086569	PRECIPITAT?
S33	2116041	CHROMATOGRAPHY
S34	697	S5(5N)S7
S35	458	S29 AND S30 AND S31 AND S32 AND S33
S36	109	S34 NOT PY>1995
S37	76	RD (unique items)
S38	43	S35 NOT PY>1995
S39	43	RD (unique items)

24/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07777015 93301593 PMID: 8100267
Compartmentalization of T lymphocytes to the site of disease: intrahepatic CD4+ T cells specific for the protein NS4 of hepatitis C virus **in patients with chronic hepatitis C.**
Minutello M A; Pileri P; Unutmaz D; Censini S; Kuo G; Houghton M; Brunetto M R; Bonino F; **Abrignani S**
Immunobiology Research Institute, Siena, Italy.
Journal of experimental medicine (UNITED STATES) Jul 1 1993, 178 (1) p17-25, ISSN 0022-1007 Journal Code: 2985109R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The adult liver is an organ without constitutive lymphoid components. Therefore, any intrahepatic T cell found in chronic hepatitis should have migrated to the liver after infection and inflammation. Because of the little information available on the differences between intrahepatic and peripheral T cells, we used recombinant proteins of the **hepatitis C virus** (**HCV**) to establish specific T cell lines and clones from liver biopsies of patients with chronic hepatitis C and compared them with those present in peripheral blood mononuclear cells (PBMC). We found that the protein nonstructural 4 (NS4) was able to stimulate CD4+ T cells isolated from liver biopsies, whereas with all the other **HCV** proteins we consistently failed to establish liver-derived T cell lines from 16 biopsies. We then compared NS4-specific T cell clones obtained on the same day from PBMC and liver of the same patient. We found that the 22 PBMC-derived T cell clones represent, at least, six distinct clonal populations that differ in major histocompatibility complex restriction and response to superantigens, whereas the 27 liver-derived T cell clones appear all identical, as further confirmed by cloning and sequencing of the T cell **receptor** (TCR) variable and hypervariable regions. Remarkably, none of the PBMC-derived clones has a TCR identical to the liver-derived clone, and even with polymerase chain reaction oligotyping we did not find the liver-derived clonotypic TCR transcript in the PBMC, indicating a preferential intrahepatic localization of these T cells. Functionally, the liver-derived T cells provided help for polyclonal immunoglobulin (Ig)A production by B cells in vitro that is 10-fold more effective than that provided by the PBMC-derived clones, whereas there is no difference in the help provided for IgM and IgG production. Altogether these results demonstrate that the protein NS4 is highly immunogenic for intrahepatic CD4+ T cells primed by **HCV** in vivo, and that there can be compartmentalization of some NS4-specific CD4+ T cells to the liver of patients with chronic hepatitis C.

24/3,AB/2 (Item 1 from file: 77)
DIALOG(R)File 77:Conference Papers Index

4492011
Supplier Accession Number: 00-01661 V28N02
Pathogenetic implication of HCV-CD81 interaction
Abrignani, S.
IRIS, Chiron Res. Cent.
1999 Annual Meeting of Institute of Human Virology 9935069 Baltimore, MD (USA) 28 Aug - 2 Sep 1999
Adv Bioscience Labs, Becton Dickinson/Pharmingen, Beckman Coulter, Immune Response Corp, Merck, Organon Teknika, Roche Boehringer Mannheim, SANYO
Institute for Human Virology, 725 West Lombard Street, Baltimore, MD 21201, USA; phone: 410-706-8614; email: valuckas@umbi.umd.edu; URL: www.ihv.org, Abstracts available. Contact IHV for price.

24/3,AB/3 (Item 2 fr[redacted] file: 77)
DIALOG(R)File 77:Conference Papers Index

4438126
Supplier Accession Number: 99-04167 V27N04
CD81 is the receptor of hepatitis C virus
Abrignani, S.
Winter Meeting of the European Society of Clinical Virology 9910166
Rotterdam (Netherlands) 7-9 Jan 1999
Roche Diagnostics, GlaxoWellcome, Abbott Diagnostics Div, Organon Teknika, Eurogentec, Bayer Bayer, Gull Diagnostics
Erasmus Medical Center Rotterdam, Institute of Virology, Room Ee1726, P.O. Box 1738, 3000 DR Rotterdam, Netherlands; phone: + 31 10 408 8066; fax: + 31 10 436 5145; URL: www.eur.nl/fgg/viro/escv, Abstracts available. No charge.
?

39/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02773335 78088346 PMID: 340586
Human cell membrane **components bound to beta2-microglobulin in T cell-type cell lines.**
Tada N; Tanigaki N; Pressman D
Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Feb 1978, 120 (2) p513-9, ISSN 0022-1767 Journal Code: 2985117R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Cell membrane components bound to beta2-microglobulin were isolated from Renex 30 (a nonionic detergent)- **solubilized** membrane materials of two human T cell-type cell lines, **MOLT - 4** and CCRF-CEM, by gel filtration and lectin affinity **chromatography** . The isolation was carried out by following the beta2-microglobulin activity by radioimmune inhibition assay. The T **cell membrane** components bound to beta2-microblogulin had a uniform molecular size of about 200,000 daltons and most of them showed an affinity to lentil lectin. The isolated membrane components were radioiodinated and examined for identity to HLA antigens by sequential **precipitation** with rabbit anti-HLA antiserum (specific to HLA large components) and with rabbit anti-beta2-microblogulin antiserum. In addition to HLA antigens, the beta2-microglobulin-bound components obtained from the **MOLT - 4** cells were found to contain certain membrane components that are the same in molecular size as the HLA large components but that are different antigenically from the HLA large components. On the other hand, the beta2-microglobulin-bound membrane components obtained from the CCRF-CEM cells were all HLA antigens. No other membrane components were involved in the binding.

39/3,AB/4 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00393630
Monoclonal antibody reactive to a unique antigen widely present on various human leukemia and lymphoma cells and methods of using same for diagnosis and treatme
Ein mit einem spezifischen, in verschiedenen menschlichen Leukamie- und Lymphomzellen vorkommender, Antigen-reagierender, monoklonaler Antikorper und dessen Ver
Anticorps monoclonal reagissant avec un antigene unique, present largement sur diverses cellules humaines de leucemie et de lymphome, et les methodes utilisant
PATENT ASSIGNEE:
HEALTH RESEARCH, INC., (522423), Roswell Park Division, 666 Elm Street, Buffalo, NY 14263, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
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LEGAL REPRESENTATIVE:
Weber, Dieter, Dr. et al (12721), Dr. Dieter Weber und Dipl.-Phys. Klaus Seiffert Patentanwalte Gustav-Freytag-Strasse 25 Postfach 6145, D-6200 Wiesbaden 1, (DE)
PATENT (CC, No, Kind, Date): EP 400464 A1 901205 (Basic)
APPLICATION (CC, No, Date): EP 90109768 900522;
PRIORITY (CC, No, Date): US 359505 890601
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12P-021/08; C12N-015/06; G01N-033/574; C07K-015/14;

ABSTRACT EP 400464 A1

A novel hybrid cell line, designated T6-1G9, for production of monoclonal antibodies specific for a unique cell surface antigen, GP20, associated with a wide variety of human lymphomas and leukemias. Hybridoma T6-1G9 was generated by fusing mouse myeloma cells with spleen cells from a BALB/c mouse that was immunized with a human leukemia antigen preparation isolated from the **cell membranes** of acute lymphoblastic leukemia cells. This invention also provides a method for producing the new monoclonal antibody designated SN7 and to diagnostic procedures using SN7 to detect various leukemias and lymphomas. Also disclosed are methods of using SN7 or reactive fragments of SN7 for the treatment of leukemia-lymphoma patients.

ABSTRACT WORD COUNT: 113

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	1253
SPEC A	(English)	EPABF1	7317
Total word count - document A			8570
Total word count - document B			0
Total word count - documents A + B			8570

39/3,AB/7 (Item 4 from file: 348)

DIALOG(R)File 348:EUROPEAN PATENTS

00337669

Derivatives of soluble T-4.

Losliche T-4 Derivate.

Derives de T-4 solubles.

PATENT ASSIGNEE:

THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, (477541), West 116th Street and Broadway, New York New York 10027, (US), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)

SMITHKLINE BECKMAN CORPORATION, (201240), P.O. Box 7929 1 Franklin Plaza, Philadelphia Pennsylvania 19101, (US), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)

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Sweet, Raymond W., 108 Edgehill Road, Bala Cynwyd, Pennsylvania 19004, (US)

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LEGAL REPRESENTATIVE:

Lawrence, John et al (60371), Barker, Brettell & Duncan 138 Hagley Road Edgbaston, Birmingham B16 9PW, (GB)

PATENT (CC, No, Kind, Date): EP 330227 A2 890830 (Basic)
EP 330227 A3 910130

APPLICATION (CC, No, Date): EP 89103297 890224;

PRIORITY (CC, No, Date): US 160348 880224

DESIGNATED STATES: AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE

INTERNATIONAL PATENT CLASS: A61K-037/02; C12N-015/13; C12N-015/00; C12P-021/02;

ABSTRACT EP 330227 A2

This invention provides a therapeutic agent capable of specifically forming a complex with human immunodeficiency virus envelope glycoprotein which comprises a polypeptide. In one embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +185 fused to the amino acid sequence from about +351 to about +369. In another embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +106 fused to the amino acid sequence from about +351 to about +369. In yet a further

embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +185.

This invention also provides a method for treating a subject infected with a human immunodeficiency virus. The method comprises administering to the subject an effective amount of a pharmaceutical composition comprising an effective amount of a therapeutic agent of the invention and a pharmaceutically acceptable carrier.

ABSTRACT WORD COUNT: 182

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	289
SPEC A	(English)	EPABF1	25033
Total word count - document A			25322
Total word count - document B			0
Total word count - documents A + B			25322

39/3,AB/8 (Item 1 from file: 349)

00302504

NOVEL CELL SURFACE RECEPTOR, ANTIBODY COMPOSITIONS, AND METHODS OF USING SAME

NOUVEAU RECEPTEUR DE SURFACE CELLULAIRE, COMPOSITIONS D'ANTICORPS ET PROCEDES UTILISANT UN TEL RECEPTEUR

Patent Applicant/Assignee:
THE SCRIPPS RESEARCH INSTITUTE,
Inventor(s):
ALTIERI Dario C,

Patent and Priority Information (Country, Number, Date):
Patent: WO 9520655 A1 19950803
Application: WO 95US666 19950118 (PCT/WO US9500666)
Priority Application: US 94189309 19940128
Designated States: AU CA FI JP NO AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 46485

English Abstract

A new class of cellular receptors extensively homologous but not identical to coagulation factors V and VIII is identified. The DNA and amino acid residue sequences of the receptor are also described. The invention also discloses methods, sequences and vectors useful in the purification and synthesis of cellular receptors of the present invention. Antibody compositions capable of immunoreacting with the receptor or with polypeptides containing the identified amino acid residue sequences and related therapeutic and diagnostic protocols are also described, as are polypeptides, compositions and methods relating to the inhibition of T lymphocyte proliferation using the antibodies disclosed herein. The receptors are also demonstrated to bind coagulation factor Xa, which binding is inhibited by various disclosed monoclonal antibodies to the receptors. The present invention also discloses polypeptides, antibodies and compositions capable of stimulating or co-stimulating lymphocyte proliferation.

French Abstract

Cette invention se rapporte a l'identification d'une nouvelle classe de recepteurs cellulaires qui sont au sens large homologues mais pas identiques aux facteurs de coagulation V et VIII. Les sequences d'ADN et de residus d'acides amines de ce recepteur sont egalement decrites. Cette invention presente egalement des procedes, des sequences et des vecteurs utiles dans la purification et la synthese de recepteurs cellulaires de

cette invention. Des compositions d'anticorps capables d'entrer en immunoreaction avec un tel recepteur ou avec des polypeptides contenant les sequences de residus d'acides amines identifies et les protocoles therapeutiques et diagnostiques apparentes sont egalement decrits, ainsi que des polypeptides, des compositions et des procedes se rapportanta l'inhibition de la proliferation de lymphocytes T au moyen des anticorps decrits ici. Ces recepteurs se sont egalement reveles capables de fixer le facteur de coagulation Xa, cette fixation etant inhibee par divers anticorps monoclonaux de ces recepteurs, ces anticorps etant egalement presentes ici. Cette invention decrit egalement des polypeptides, des anticorps et des compositions capables de stimuler ou de co-stimuler la proliferation de lymphocytes.

39/3,AB/13 (Item 6 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00235551
MURINE MONOCLONAL ANTIBODY (5c8) RECOGNIZES A HUMAN GLYCOPROTEIN ON THE SURFACE OF T-LYMPHOCYTES
ANTICORPS MONOCLONAL MURIN (5c8) CAPABLE DE RECONNAITRE UNE GLYCOPROTEINE HUMAINE SUR LA SURFACE DE LYMPHOCYTES T
Patent Applicant/Assignee:
THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,
LEDERMAN Seth,
CHESS Leonard,
YELLIN Michael J,
Inventor(s):
LEDERMAN Seth,
CHESS Leonard,
YELLIN Michael J,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9309812 A1 19930527
Application: WO 92US9955 19921116 (PCT/WO US9209955)
Priority Application: US 91728 19911115
Designated States: AU CA JP US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE
Publication Language: English
Fulltext Word Count: 42611

English Abstract
This invention provides a monoclonal antibody capable of binding to a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916. This invention also provides an isolated protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC No. HB 10916. This invention further provides an isolated nucleic acid molecule encoding a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC No. HB 10916. This invention also provides a human CD4- T cell leukemia cell line designated D1.1 having ATCC Accession No. CRL 10915 capable of constitutively providing contact-dependent helper function to B cells.

French Abstract
L'invention concerne un anticorps monoclonal capable de se lier a une proteine qui est specifiquement reconnue par l'anticorps monoclonal 5c8 produit par l'hybridome ayant le ndegrees HB 10916 aupres de l'ATCC. Cette invention concerne egalement une proteine isolee qui est specifiquement reconnue par l'anticorps monoclonal 5c8 produit par l'hybridome ayant le ndegrees HB 10916 aupres de l'ATCC. Cette invention concerne egalement une molecule d'acide nucleique isolee codant une proteine qui est specifiquement reconnue par l'anticorps monoclonal 5c8 produit par l'hybridome ayant le ndegrees HB 10916 aupres de l'ATCC. Cette invention concerne egalement une lignee de cellules leucemiques de lymphocytes T CD4- designee D1.1 ayant le ndegrees CRL 10915 aupres de l'ATCC pouvant avoir une fonction auxiliaire dependant du contact sur les

cellules B.

39/3,AB/15 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00213290
HUMAN RETROVIRUS RECEPTOR AND DNA CODING THEREFOR
RECEPTEUR DE RETROVIRUS HUMAIN ET ADN LE CODANT
Patent Applicant/Assignee:
NEW YORK UNIVERSITY,
Inventor(s):
MERUELO Daniel,
YOSHIMOTO Takayuki,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9210506 A1 19920625
Application: WO 91US9382 19911213 (PCT/WO US9109382)
Priority Application: US 90950 19901214
Designated States: AT AU BE CA CH DE DK ES FR GB GR IT JP LU MC NL SE
Publication Language: English
Fulltext Word Count: 31695

English Abstract
A human protein molecule termed H13 has strong sequence homology to murine retrovirus receptor proteins and encodes a human retrovirus receptor. DNA encoding the H13 protein, cells transformed and transfected with this DNA and antibodies specific for H13 are disclosed. The H13 protein or its functional derivative can be used for preventing or treating retrovirus infection by administration to a subject of the H13 protein or a functional derivative thereof, or an anti-H13 antibody. Transgenic animals, useful as animal models for diagnosis or therapy of human retrovirus infections, are made by transfecting embryonic cells with the H13-encoding DNA. A chimeric retrovirus receptor protein comprises the H13 sequence, having substituted therein, amino acid residues encoding a murine retroviral receptor. Expression of the chimeric receptor in human cells allows infection or retrovirus-mediated gene transfer with murine retroviruses, which provides an extra measure of safety for in vivo gene therapy. DNA encoding the chimeric retrovirus receptor protein, cells transformed with this DNA, and methods for rendering a cell susceptible to infection by a retrovirus normally incapable of infecting that cell are disclosed.

French Abstract
Molecule de proteine humaine appelee H13 presentant une forte homologie de sequences avec des proteines receptrices de retrovirus murines et codant un recepteur de retrovirus humain. L'invention concerne aussi de l'ADN codant la proteine H13, des cellules transformees et transfectees avec cet ADN ainsi que des anticorps specifiques de la proteine H13. La proteine H13, ou son derive fonctionnel, peut etre utilisee dans la prevention ou le traitement d'infections retrovirales par administration a un sujet de la proteine H13 ou d'un derive fonctionnel de celle-ci, ou bien d'un anticorps anti-H13. On obtient des animaux transgeniques utiles comme modeles animaux de diagnostic ou de therapie d'infections retrovirales humaines par transfection de cellules embryonnaires avec l'ADN codant la H13. Une proteine de retrovirus chimerique comprend la sequence de H13 dans laquelle sont substitues le reste d'acides amines codant un recepteur retroviral murin. L'expression du recepteur chimerique dans des cellules humaines permet l'infection ou le transfert de genes induits par retrovirus a l'aide de retrovirus murins, ce qui constitue une mesure supplementaire de securite pour une therapie genique in vivo. L'invention concerne egalement de l'ADN codant la proteine receptrice de retrovirus chimerique, des cellules transformees a l'aide de cet ADN, et des procedes permettant de rendre une cellule susceptible d'etre infectee par un retrovirus incapable d'infecter cette cellule.

39/3,AB/16 (Item 9 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00196557
PEPTIDE INHIBITOR OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION BLOCKS VIRUS INTERACTIONS WITH A NOVEL CELLULAR RECEPTOR
EFFET DE BLOCAGE DE L'INHIBITEUR PEPTIDIQUE DE L'INFECTION CAUSEE PAR LE VIRUS DE L'IMMUNODEFICIENCE HUMAINE SUR LES INTERACTIONS ENTRE LE VIRUS ET UN NOUVEAU RECEPTEUR CELLULAIRE
Patent Applicant/Assignee:
ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND,
Inventor(s):
HENDERSON Lee Alan,
COY David H,
GARRY Robert Francis Jr,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9113911 A1 19910919
Application: WO 91US1549 19910307 (PCT/WO US9101549)
Priority Application: US 90137 19900309; US 9016 19901002; US 90652 19901212
Designated States: AT AU BE CA CH DE DK ES FR GB GR IT JP LU NL SE
Publication Language: English
Fulltext Word Count: 10284
English Abstract
The present invention relates to methods of inhibiting HIV-mediated cell killing which comprises inhibiting the interaction between the CS3 region and viral gp41 and its receptor on the surface of lymphocytes. The invention provides for methods which employ peptides, peptide derivatives, or antibodies to inhibit the CS3/CS3 receptor interaction. In addition, the invention also relates to the CS3 receptor. The present invention is based in part on the discovery that a CS3 specific cellular receptor is widely distributed on human lymphocytes and forms a 108 kd complex with CS3-HSA peptide conjugate. It was further discovered that CS3 peptide effectively blocks HIV mediated cell infection and killing. Therefore, the present invention provides for methods of treatment and prophylaxis of HIV infection as well as a means for better understanding the physiology of acquired immunodeficiency syndrome (AIDS).

French Abstract
Procedes d'inhibition de la destruction de cellules due au VIH consistant a inhiber l'interaction entre la region CS3 de la glycoproteine gp41 virale et son recepteur a la surface des lymphocytes. Les procedes proposes sont l'emploi de peptides, de derives peptidiques ou d'anticorps visant a inhiber l'interaction CS3/recepteur CS3. L'invention porte egalement sur le recepteur CS3. L'invention se fonde en partie sur la decouverte qu'un recepteur cellulaire specifique CS3 est largement reparti sur les lymphocytes humains et forme un complexe de 108 kd avec le conjugue peptidique CS3-HSA. On a aussi decouvert que le peptide CS3 bloque effectivement l'infection et la destruction de cellules dues au VIH. En consequence, on propose des procedes de traitement et de prophylaxie de l'infection par le VIH ainsi qu'un moyen de mieux comprendre la physiologie du syndrome d'immunodeficience acquise (SIDA).

39/3,AB/23 (Item 16 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00161763
DERIVATIVES OF SOLUBLE T-4
DERIVES DE T-4 SOLUBLE
Patent Applicant/Assignee:
THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,

SMITHKLINE BECKMAN CORPORATION,
Inventor(s):
MADDON Paul J,
AXEL Richard,
SWEET Raymond W,
ARTHOS James,
Patent and Priority Information (Country, Number, Date):
Patent: WO 8908143 A1 19890908
Application: WO 89US762 19890224 (PCT/WO US8900762)
Priority Application: US 88348 19880224
Designated States: AU DK FI HU JP KR NO
Publication Language: English
Fulltext Word Count: 28652

English Abstract
This invention provides a therapeutic agent capable of specifically forming a complex with human immunodeficiency virus envelope glycoprotein which comprises a polypeptide. In one embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +185 fused to the amino acid sequence from about +351 to about +369. In another embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +106 fused to the amino acid sequence from about +351 to about +369. In yet a further embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in Figure 6 from about +3 to about +185. This invention also provides a method for treating a subject infected with a human immunodeficiency virus. The method comprises administering to the subject an effective amount of a pharmaceutical composition comprising an effective amount of a therapeutic agent of the invention and a pharmaceutically acceptable carrier.

French Abstract
Cette invention concerne un agent therapeutique capable de former d'une maniere specifique un complexe avec une glycoproteine de l'enveloppe d'un virus d'immunodeficience humaine et qui comprend un polypeptide. Dans un mode de realisation de l'invention, la sequence d'acides amines du polypeptide comprend la sequence d'acides amines illustree dans la figure 6 depuis +3 jusqu'a +185 environ fusionnee a la sequence d'acides amines allant de +351 environ jusqu'a +369 environ. Dans un autre mode de realisation de l'invention, la sequence d'acides amines du polypeptide comprend la sequence d'acides amines illustree dans la figure 6 depuis +3 environ jusqu'a +106 environ fusionnee sur la sequence d'acides amines allant de +351 environ jusqu'a +369 environ. Dans un autre mode de realisation de l'invention, la sequence d'acides amines du polypeptide comprend la sequence d'acides amines illustree dans la figure 6 depuis +3 environ jusqu'a +185 environ. Cette invention concerne egalement un procede de traitement d'un sujet infecte par le virus d'immunodeficience humaine. Le procede consiste a administrer au sujet une quantite efficace d'une composition pharmaceutique comprenant une quantite efficace d'un agent therapeutique de l'invention et un vehicule pharmaceutiquement acceptable.

39/3,AB/34 (Item 8 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

3473975
Derwent Accession: 1992-200315
Utility
REASSIGNED
C/ Therapeutic and diagnostic methods using soluble T cell surface molecules; IMMUNOSPECIFIC BINDING
Inventor: Kung, Patrick C., Lexington, MA
Ip, Stephen H., Sudbury, MA

Brown, Michael C., Wayland, MA
MacKeen, Linda A., Elkins Park, PA
Assignee: T Cell Diagnostics, Inc. (02), Cambridge, MA
T Cell Diagnostics Inc (Code: 32378)
Examiner: Saunders, David (Art Unit: 182)
Law Firm: Pennie & Edmonds

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5292636	A	19940308	US 89434398	19891109
CIP	Abandoned			US 88254551	19881006
CIP	US 5006459	A	19910509	US 8720819	19870302
CIP	Abandoned			US 86846230	19860319
Priority				US 89434398	19891109
				US 88254551	19881006
				US 8720819	19870302
				US 86846230	19860319

Abstract:
The present invention is directed to the measurement of soluble T cell growth factor receptors, soluble T cell differentiation antigens, or related soluble molecules or fragments thereof, and the use of such measurements in the diagnosis, staging, and therapy of diseases and disorders. Specific embodiments involve the diagnosis and monitoring of therapy using absolute values of such soluble molecules. Further embodiments involve detecting a change in the levels of such soluble molecules, in the diagnosis and therapy of diseases and disorders. In specific embodiments, measurements of interleukin-2 receptor levels can be made to detect lung cancer, or to stage squamous cell lung carcinoma. In other embodiments, detection of increases in both soluble IL2R and creatinine in the body fluid of a transplant patient can be used to differentially diagnose renal allograft rejection from infection. The invention is also directed to methods for measurement of soluble CD4 antigens, which measurements can be used, in a specific embodiment, to diagnose a state of immune activation, to diagnose rheumatoid arthritis, to monitor therapeutic efficacy (e.g. of AIDS treatments), or to stage adult T cell leukemia in a patient. In another aspect, the invention relates to the detection, staging, and monitoring of therapy of diseases and disorders by measuring a plurality of soluble T cell markers.
Document type: C REASSIGNED

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DIALOG(R)File 654:US PAT.FULL.

3364654
Derwent Accession: 1989-130019
Utility
EXPIRED
C/ **Antibodies to natural killer cell and non-specific cytotoxic cell receptor and target cell antigens; MONOCLONAL ANTIBODIES**
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Main Patent	US 5194593	A	19930316	US 90592685	19901003
Division	Pending			US 87109730	19871016

Priority	US 90592685	19901003
	US 87109730	19871016

Abstract:
The present invention encompasses a receptor molecule present on the surface of natural killer cells (NK cells) and non-specific cytotoxic cells (NCC) which is involved in non-specific lysis of target cells bearing an antigen recognized by the receptor molecule, an antigen common to the surface of cells recognized and lysed by natural killer cells, monoclonal and heterologous antibodies which bind to the receptor and target cell antigen(s) which are useful in their identification and purification, and methods for altering NK cell-mediated lysis of target cells.

The monoclonal antibodies (mAbs) of the present invention were prepared by cell fusions between spleen cells from mice immunized with either non-specific cytotoxic cells (NCC) from catfish (anti-receptor antibodies) or NC-37 human lymphoblastoid B-cells, which are susceptible to lysis by NK cells, and myeloma cells. Eight distinct mAbs have been characterized, four directed against the NK/NCC target cell receptor and four against the NK/NC cell antigen. As used in the present invention "NK cells" will be used to include both natural killer cells of mammalian origin and non-specific cytotoxic cells of fish origin, unless specifically stated otherwise. Two of the mAbs directed against the receptor and two of the mAbs against the NK target cell antigen are able to significantly inhibit NK lysis at low mAb concentrations, as assessed by inhibition of cellular toxicity. Two of the mAbs directed against the receptor and two of the mAbs against the NK target cell antigen bind to the antigen(s) but do not inhibit lysis.
Document type: C EXPIRED

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3272718
Derwent Accession: 1989-250337
Utility
C/ **Derivatives of soluble T-4; POLYPEPTIDES AGAINST HUMAN IMMUNODEFICIENCY VIRUS**
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Priority				US 88160348	19880224
				US 87114244	19871023
				US 86898587	19860821

Abstract:
This invention provides a therapeutic agent capable of specifically forming a complex with human immunodeficiency virus envelope glycoprotein

which comprises a polypeptide. In one embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in FIG. 6 from about +1 to about +185 fused to the amino acid sequence from about +353 to about +371. In another embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in FIG. 6 from about +1 to about +106 fused to the amino acid sequence from about +353 to about +371. In yet a further embodiment of the invention, the amino acid sequence of the polypeptide comprises the amino acid sequence shown in FIG. 6 from about +1 to about +185.

This invention also provides a method for treating a subject infected with a human immunodeficiency virus. The method comprises administering to the subject an effective amount of a pharmaceutical composition comprising an effective amount of a therapeutic agent of the invention and a pharmaceutically acceptable carrier.

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(54) Monoclonal antibody reactive to a unique antigen widely present on various human leukemia and lymphoma cells and methods of using same for diagnosis and treatment.

(57) A novel hybrid cell line, designated T6-1G9, for production of monoclonal antibodies specific for a unique cell surface antigen, GP20, associated with a wide variety of human lymphomas and leukemias. Hybridoma T6-1G9 was generated by fusing mouse myeloma cells with spleen cells from a BALB/c mouse that was immunized with a human leukemia antigen preparation isolated from the cell membranes of acute lymphoblastic leukemia cells. This invention also provides a method for producing the new monoclonal antibody designated SN7 and to diagnostic procedures using SN7 to detect various leukemias and lymphomas. Also disclosed are methods of using SN7 or reactive fragments of SN7 for the treatment of leukemia-lymphoma patients.

EP 0 400 464 A1

MONOCLONAL ANTIBODY REACTIVE TO A UNIQUE ANTIGEN WIDELY PRESENT ON VARIOUS HUMAN LEUKEMIA AND LYMPHOMA CELLS AND METHODS OF USING SAME FOR DIAGNOSIS AND TREATMENT

The invention described herein was made in the course of work done under the support of a PHS GRANT CA 19304 awarded by the National Cancer Institute.

TECHNICAL FIELD

This invention relates generally to hybridoma cell lines and monoclonal antibodies produced therefrom. More specifically, this invention relates to a novel hybridoma cell line that produces a monoclonal antibody specific for a unique antigen associated with a wide variety of human leukemias and lymphomas, to the monoclonal antibody generated from the hybridoma cell line, to the antigen isolated from human leukemia cells and lymphoma cells, and to methods of using the monoclonal antibody, in whole or in part, for the diagnosis and therapy of various human leukemia-lymphomas.

DESCRIPTION OF THE PRIOR ART

Kohler and Milstein reported the first successful use of cell hybridization technology in generating continuously growing hybrid cell lines (called "hybridomas") that produce monoclonal antibodies [Nature 256:495-497 (1975)]. Monoclonal antibodies (mAbs) are homogeneous antibodies which exhibit selective binding to a single antigenic determinant.

MAbs have significant advantages over conventional antisera with respect to specificity and availability. This is particularly so for mAbs directed to cell surface antigens [see e.g., Curr. Top. Dev. Biol. 14:1-32 (1980)].

Since 1975, much effort has been exerted by a number of investigators to generate hybridomas that produce mAbs directed to human leukemia-lymphoma (HLL) associated cell surface antigens. Several researchers, including the inventor herein, have successfully generated such anti-HLL mAbs. These mAbs include those directed to common ALL (acute lymphoblastic leukemia) antigen termed CALLA (CD10; a neutral endopeptidase), human thymyus-leukemia antigens (CD1), T leukemia antigens and a lymphocytic-myelocytic leukemia associated antigen termed GP160.

Most, if not all, of these mAbs, except for those generated by the inventor herein, were produced by the conventional approach, i.e., by using intact HLL cells for immunizing mice to provide spleen cells for cell fusion. Despite the success described above, it is still difficult to generate anti-HLL mAbs by the conventional approach. This is probably because HLL-associated cell surface antigens are, in general, relatively minor cell surface components and poorly immunogenic as compared to other known major cell surface components such as HLA class I and class II antigens.

The use of purified, isolated cell membrane antigens, rather than intact cells for immunizing mice should result in the production of mAbs with a greater affinity for relatively weak immunogens, e.g., HLL associated cell surface antigens, because of the absence of antigenic competition with strong immunogens, e.g., HLA class I and class II antigens, during the immune response. Further, such use may give rise to a new group of mAbs that have not been obtained by using whole cells, i.e., those directed to antigenic determinants which are unable to induce an immune response when they are on intact cells.

In this regard, the present inventor previously developed a novel procedure for isolating relatively large quantities (submilligrams) of immunologically active HLL associated cell membrane antigens [Cancer Research 41:2973-2976 (July 1981); J. Immunol. 127:2580-2588 (1981)]. Using such antigen preparations, several mAbs directed toward several different HLL associated antigens have been generated [e.g., Proc. Natl. Acad. Sci. USA 80:845-849 (1983); J. Immunol. 132:2089-2095 (1984); Proc. Natl. Acad. Sci. USA 83:7898-7902 (1986)]. However, these antigens are different from the unique antigen reported in this patent application.

During the past decade, remarkable progress has been made in the classification and characterization of HLL. MAbs have made a great contribution to this progress. Furthermore, mAbs have been extensively used for the diagnosis and follow-up of HLL patients.

However, successful utilization of mAbs for th rapy of HLL has been very limited. An ideal mAb for

such purposes will be one that reacts with 100% of the malignant cells for all HLL specimens but does not react with any of the normal cells or normal tissues.

This inventor has produced and characterized many mAbs which show various degrees of reactivity with HLL cells. Most of these anti-HLL mAbs were generated by using antigen preparations isolated from cell membranes using the above-mentioned procedure. Some of the mAbs generated showed remarkably high tumor specificity. However, because of their narrow specificity, some of these highly selective anti-HLL mAbs have only limited therapeutic utility. For instance, the mAb, designated SN1, reacts only with T acute lymphoblastic leukemia (T ALL) cells among many malignant and nonmalignant cell and tissue specimens tested. Although this antibody may be suitable for the in vivo therapy of T ALL patients, SN1 would have only limited therapeutic utility because T ALL has a low incidence among the general population. Therefore, the need remains for mAbs which strongly react with many different types of HLL but show a relatively low reactivity with normal cells, particularly normal bone marrow progenitor cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the graphic results of FACS analysis of the SN7 monoclonal antibody with selected human cell specimens.

FIG. 2 depicts the results of SDS/PAGE analysis of the SN7 antigen.

DISCLOSURE OF THE INVENTION

The present invention provides a new mAb designated SN7 and/or a reactive fragment of SN7 which binds or reacts with a wide variety of human leukemia-lymphoma cells including one or more, and preferably a plurality and more preferably a majority of the following leukemia lymphoma cell specimens: human B chronic lymphocytic leukemia cells; B prolymphocytic leukemia cells; Hairy cell leukemia cells; non-T acute lymphoblastic leukemia cells; acute myelocytic leukemia cells; acute myelomonocytic leukemia cells; acute monocytic leukemia cells; chronic myelocytic leukemia cells and non-Hodgkin's lymphoma cells.

This invention further provides conjugates of SN7 and/or a reactive fragment of SN7, through direct or indirect attachment or complexation, with one or more compounds including, but not limited to the following: cytotoxic agents, drugs, toxins or fragments thereof, hormones, enzymes, liposomes, radioactive agents, dyes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

As is well known in the art, a "reactive fragment" includes any antigen-binding fragment of an IgG molecule including Fab, $F(ab')_2$ or Fv fragments.

The present invention further provides a new hybridoma cell line designated T6-1G9 which is generated by fusing mouse myeloma cells with spleen cells from a mouse or other suitable animal immunized with a human non-T leukemia antigen preparation isolated from cell membranes of human acute lymphoblastic leukemia cells. The hybridomas thus produced are screened for those with culture supernatants containing antibody which give selective binding to HLL cells. The desired hybridomas are subsequently cloned and characterized.

This invention still further provides a method for preparing mAb SN7 or a reactive fragment of SN7 which comprises culturing hybridoma cell line T6-1G9 in a suitable medium and recovering the mAb or reactive fragment from the culture supernatant of said hybridoma cell line. Alternatively, mAb SN7 or a reactive fragment of SN7 may be generated by injecting hybridoma cell line T6-1G9 into an appropriate animal and recovering the antibody or reactive fragment from the malignant ascites or serum of the animal so injected.

This invention is further directed to the novel antigen defined by mAb SN7 and to diagnostic and therapeutic methods employing mAb SN7 in the treatment of various human leukemias and lymphomas.

Mab, SN7, was tested against a variety of cultured and uncultured human cell specimens using a cellular radioimmunoassay and/or FACS analysis. Based on the information obtained from this analysis, SN7 was shown to bind or react with uncultured human B chronic lymphocytic leukemia cells; B prolymphocytic leukemia cells; Hairy cell leukemia cells; non-T acute lymphoblastic leukemia cells; acute myelocytic leukemia cells; acute myelomonocytic leukemia cells; acute monocytic leukemia cells; chronic myelocytic leukemia cells and non-Hodgkin's lymphoma cells. SN7 did not react with any T acute

lymphoblastic leukemia cell specimens test d. The results of the reactivity with various cultured human cell lines generally agreed with the above-mentioned reactivity with the uncultured cell specimens.

The reactivity of SN7 with several different normal (or nearly normal) bone marrow specim ns was either not significant or only weakly positive for a minor population of some bone marrow specimens. The bone marrow specimens used were derived from several different leukemia-lymphoma patients in remission. Of normal peripheral blood cells, T cells, granulocytes, erythrocytes and platelets showed no significant reactivity with SN7. ("No significant reactivity" is defined as that which is undetectable using a cellular radioimmunoassay (RIA), because the level of activity detected cannot be distinguished over usual background control levels.) However, SN7 showed varying degrees of weak reaction with a subpopulation of normal peripheral blood B cells and monocytes. The degree of the reactivity shown varied depending upon the donors of the peripheral blood.

SN7 was found to be an IgG1-k antibody. The molecular weight of the cell surface antigen defined by SN7 was determined to be approximately 20,000. The discovery of an antigen having such a low molecular weight is considered to be unusual when compared to those molecular weights of other known cell surface antigens on human hematopoietic cells. This antigen was designated GP20 and is believed to be a totally new antigen which is expressed on the cell surface of a wide variety of HLL cells.

In addition, mAb SN7 has been shown to be sufficiently stable to undergo known conjugation procedures with other agents, such as the A chain of ricin, a plant toxin. The conjugate has demonstrated the ability to selectively kill HLL cells which express GP20, the antigen defined by SN7. Such procedures include covalently attaching or complexing the mAb or a fragment thereof to the agent or drug of interest either directly or indirectly using a suitable linking agent. Other mAbs, SN1, SN2, SN5 and SN6, also identified by the inventor herein, when conjugated with the ricin A-chain (RA), have demonstrated highly specific killing capability for leukemia cells as reported in Cancer Research 44:259-264 (1984), Proc. Natl. Acad. Sci. USA 84:3390-3394 (1987), and Cancer Research 48:4673-4680 (1988), the disclosures of which are incorporated by reference herein.

Numerous methods have been employed during recent years for attaching or conjugating a variety of molecules to various sites on antibodies and in particular monoclonal antibodies directed against any desired target antigen. One such method is disclosed in U.S. Patent 4,671,958, the disclosure of which is incorporated by reference herein. Numerous bioactive agents may be conjugated to the mAb in accord with the present invention including cytotoxic agents, drugs, toxins or toxin fragments, hormones, enzymes, liposomes, radioisotopes, dyes, photodynamic agents, or other antibodies including anti-idiotype antibodies, chimeric antibodies and monoclonal antibodies or fragments of such antibodies. In addition, the mAb or fractions of these mAbs may be incorporated into other matrices for use in separation schemes which are based upon antibody-antigen reactions. A multitude of known carrier or conjugating agents is disclosed in U.S. Patent 4,671,958 and any of these agents would be suitable for binding to the antibodies disclosed herein or to active fragments of the antibody disclosed herein, namely $F(ab')_2$, Fab or Fv fragments without significant loss of antibody activity.

In addition, the antibody of the present invention may be made cytotoxic in a compliment-mediated cytolysis by adding rabbit anti-mouse IgG antibodies.

The foregoing and additional advantages and characterizing features of the present invention will become clearly apparent upon a reading of the ensuing detailed description together with the included examples.

DETAILED DESCRIPTION OF THE INVENTION

Materials and Methods

The monoclonal antibodies exhibiting utility in the present invention were prepared generally following the procedures of Kohler and Milstein as reported in Nature, 256:495-97 (1975).

The details of the process are well known in the art. Briefly, the process involv s injecting a mouse, rat, or other suitable animal, with an antigen. The animal is subsequently sacrificed and cells from its spleen are fused with cells of continuously replicating tumor cells, e.g., myeloma or lymphoma cells. After fusion, three cell types remain in culture: splenocytes, myeloma cells and hybrids. The splenocytes and myeloma cells die off and the hybrid cells begin to double every 24-48 hours. The result is the production of a hybrid cell

line, hybridoma, that reproduces in vitro. The population of hybridomas are screened to identify individual classes each of which secrete a single antibody specific for a desired antigen.

The antigen preparation was isolated from cell membranes of leukemia cells derived from a patient with non-T/non-B type (B-lineage) ALL. The procedures used were recently described in Proc. Natl. Acad. Sci. USA, 83:7898-7902 (1986). The procedures were based on a modification of an earlier method for isolating human T leukemia antigen preparations as disclosed in J. Immunol., 127:2580-2588 (1981); Proc. Natl. Acad. Sci. USA, 80:845-859 (1983); and J. Immunol., 132:2089-2095 (1984), the disclosures of which are incorporated by reference herein.

EXPERIMENTAL EXAMPLE

Production of Monoclonal Antibodies

A. Leukemia Antigen Preparation

The immunizing antigen preparation was produced as follows: Cell membranes of leukemia cells derived from a patient with non-T/non-B type (B-lineage) ALL were prepared by mechanical disruption of the cells followed by differential centrifugations of the disrupted cells. The membrane proteins were solubilized by deoxycholate treatment in the presence of recrystallized iodoacetamide (final concentration, 5 mM) and fractionated by affinity chromatography on serially connected columns of Lens culinaris lectin (LcH) and Ricinus communis lectin (RCA). The LcH-bound and RCA-bound glycoconjugates (mostly glycoproteins) were individually eluted, combined and subjected to passive immunoaffinity chromatography; i.e., the fractions were passed through three serially-connected immunoadsorbent columns. The immunoadsorbents consist of affinity-purified rabbit anti-human β_2-microglobulin antibodies coupled to Sepharose CL-4B, rabbit anti-human normal B cell antibodies coupled to Sepharose CL-4B and rabbit anti-human normal peripheral blood lymphocyte antibodies coupled to Sepharose CL-4B. Materials in the pass-through fractions from the above three-columns were pooled, concentrated and the passive immunoaffinity chromatography was repeated once.

B. Immunization and Somatic Cell Hybridization

1. Immunization

A mouse was immunized subcutaneously (s.c.) with 30 µg of the cell membrane antigen preparation in 0.12 ml of 10 mM Tris-HCl buffer, pH 8.0, containing 0.3 percent deoxycholate (Tris-DOC buffer) mixed with an equal volume of Freund's complete adjuvant. In addition, 2×10^9 Bordetella pertussis bacteria were injected s.c. A booster immunization was carried out by injecting s.c. 15µg of the antigen preparation mixed with Freund's incomplete adjuvant. Another booster immunization was performed by injecting i.p./i.v. (intraperitonealy/intravenously) 60µg (in 0.1 ml of Tris-DOC buffer) of the antigen preparation mixed with 0.4 ml of saline. The spleen was taken for the cell fusion 3 days after the last immunization.

2. Cell Hybridization

For the cell fusion, spleen cells (1×10^8 cells) derived from the immunized mouse were fused with P3/NS1/1-Ag4-1 (abbreviated as NS-1) murine myeloma cell line (4×10^7 cells) using polyethylene glycol [Nature 226:550-552 (1977)]. The NS-1 : myeloma cell line was obtained from the Cell Distribution Center of the Salk Institute, San Diego, CA. The fused cells were washed and centrifuged. The washed cell pellet was suspended in 200 ml of hypoxanthine-aminopterine-thymidine (HAT) medium and 1 ml of the cell suspension was placed in each of the wells of eight 24-well (3.5 ml capacity per well) tissue culture plates (Linbro Division, Flow Laboratories, Inc.). Each well already contained 2×10^5 cells of BALB/c mice

peritoneal exudate cells in 0.2 ml of HAT medium as feeder cells. The cells were cultured in a CO_2 (5 percent) incubator at 37° C. One-half of the culture medium of each well was replaced with fresh HAT medium twice a week. On day 12 after the cell fusion, the initial screening test was carried out using a radioimmunoassay to identify the cultured supernates containing the desired antibody.

3. Screening and Cloning of Hybridoma

Monoclonal anti-leukemia antibodies in the culture supernatants of the hybridomas were screened by a radioimmunoassay (see below). Cloning of hybridomas was carried out by propagating from a single cell by means of limiting dilution [Selected Methods in Cellular Immunology (B.B. Mischell and S.M. Shiigi, Eds.) pp. 351-372; Freeman and Company, San Francisco, 1980]. To ensure the monoclonality of the clone, the hybridoma clone resulting from the first cloning was subjected to recloning.

Characterization of SN7 Reactivity

A. Microscale Radioimmunoassay

It should be noted that Fc receptors on the target cells are blocked with human IgG during the assay.

1. Preparation of F(ab')$_2$ of Affinity-Purified Goat Anti-Mouse IgG Antibodies

To establish an efficient, sensitive radioimmunoassay, the F(ab')$_2$ fragment of affinity-purified goat anti-mouse IgG antibodies was first prepared as follows: The IgG of goat anti-mouse IgG antiserum was digested with pepsin [Arch. Biochem. Biophys. 89:230-244 (1960); Immunochemistry 13:407-415 (1976)] and the digest was fractionated on a Sephadex G-150 column [Immunochemistry 13:407-415 (1976)]. The resultant F(ab')$_2$ fragment was passed through an immunoadsorbent column prepared with Sepharose CL-4B conjugated with human IgG. This treatment was carried out to remove any F(ab')$_2$ components which react with antigenic determinants common to mouse IgG and human IgG. This treatment is important when the F(ab')2 is tested in the presence of human cells (see below). The unbound F(ab')2 was applied to an immunoadsorbent column prepared with mouse IgG coupled to Sepharose CL-4B and the column washed. The bound goat F(ab')$_2$ was eluted with 0.1 M glycine-HCl buffer, pH 2.6, containing 0.2 M NaCl, 1 mM EDTA and 0.03 percent NaN_3. This eluted F(ab')$_2$ was found to consist of only the monomer form and not to contain any significant amount of aggregates of F(ab')$_2$ as demonstrated by gel filtration on a Sepharose CL-6B column. This F(ab')$_2$ fragment preparation of specific goat antibodies directed to mouse IgG [designated as F(ab')$_2$-GαMIgG] was used for radioimmunoassay after being radiolabeled with carrier-free ^{125}I by the IODO-GEN method. [Biochemistry 17:4807-4817 (1978); Biochem. Biophys. Res. Commun. 80:849-857 (1978); J. Immunol. 127:2580-2588 (1981)]. The quantitative binding test using this ^{125}I-F(ab')$_2$-GαMIgG against purified mouse IgG showed that below ng quantities of mouse IgG antibodies could be determined by using this radiolabeled antibody reagent. The present antibody reagent was found to react with mouse IgM almost as efficiently as with mouse IgG.

2. Radioimmunoassay

Using the ^{125}I-F(ab')$_2$-GαMIgG, a microscale radioimmunoassay was employed to determine the reactivity of mAbs with various cells. In a typical assay, triplicate 20-μl aliquots of various dilutions of culture fluids or ascites of hybridomas and 2-10 x 10^5 cells in 10 μl of Hepes (pH 7.3) buffer containing 0.1 percent of human IgG were incubated in individual wells of 96-well microtiter plates (Cooke Engineering Co.) for 60 min at 4° C with continuous shaking. The Hepes buffer consisted of RPMI 1640 medium containing 25 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonate (Hepes), 0.5 percent bovine serum albumin, Trasylol (50 kallikrein units/ml) and 0.1 percent NaN_3. The human IgG is added to the Hepes buffer to minimize non-immunospecific binding (both biospecific, e.g., Fc receptor, and non-biospecific) of mouse antibodies and ^{125}I-labeled F(ab')$_2$-GαMIgG to the cells during the radioimmunoassay. In addition, the microtiter plate wells

were treated before use with the Hepes buffer.

The mixtures were centrifuged at 500 x g and 4° C for 10 minutes and the pelleted cells washed three times. Approximately 2ng (3-5 x 10^4 cpm) of the ^{125}I-F(ab′)2 in 10 µl of Hepes buffer were added to each washed pellet and the reaction mixtures incubated with shaking and washed as described above. The radioactivity in the washed pellet was determined in a gamma-ray spectrometer. When platelets are used as targets, the radioimmunoassay is carried out in conical polypropylene tubes (1.5 ml size) instead of microtiter plates because it is necessary to precipitate platelets at significantly higher centrifugal force, (e.g., 2500 x g).

Three different kinds of controls were included for individual cell specimens. In the control sample (in triplicate) for hybridoma culture supernate, the culture supernate was replaced with either culture medium, culture medium containing mouse IgG (10 µg/ml) or culture medium containing mouse IgM (10µg/ml). No significant difference was observed in the amounts of radioactivity detected in the three controls and the average value of these controls was subtracted from the radioactivity of test samples. As the control sample (in triplicate) for the dilutions of hybridoma ascites, the corresponding dilutions of appropriate control murine ascites containing mouse Ig of the same isotype as the hybridoma antibody were included [Proc. Natl. Acad. Sci. USA 80:845-849 (1983)].

A microscale radioimmunoassay using a microtiter plate is preferred since it is very sensitive, reproducible and objective. Further, a single test using this assay, permits the determination of 100 samples, in triplicate.

B. FACS Analysis

FACs analysis was carried out as follows. Cells (1.5 to 2 million) were suspended in 10 to 20 µl of RPMI 1640 medium containing 25 mM Hepes, 0.1% human IgG, 0.5% bovine serum albumin, 2 mM EDTA, Trasylol (50 kallikrein units/ml), and 0.1% NaN_3 (Buffer A) and allowed to stand for 30 min at 4° C. Then, the cells were incubated with 100µl of hybridoma culture supernatant or an isotype-matching control mouse IgG solution (10 µg/ml) for 90 min at 4° C. After three washes with cold phosphate-buffered saline, the cells were incubated with fluorescein-conjugated F(ab′)2 fragment of sheep anti-mouse Ig (SaMIg) (Sigma, St. Louis, MO) for 90 min at 4° C. The incubated cells were washed 3 times with cold phosphate-buffered saline and suspended in 25 mM Hepes buffer, pH 7.2, in RPMI 1640 medium containing 2.5 mM EDTA and 5% fetal bovine serum. To fix the cells, 1 ml of the cell suspension was mixed with an equal volume of a 16% formaldehyde solution in RPMI 1640 medium. The fixed cells were kept in the cold room protected from the light until analyzed by FACS using a Becton Dickinson FACS 440. Each sample containing at least 10,000 cells was analyzed using the log amplification mode. Negative controls were target cells labeled with control mouse IgG and FITC-labeled second antibody, i.e., $F(ab')_2$-SaMIg.

C. Radioimmunoprecipitation and Sodium Dodecyl Sulfate Poly-acrylamide Gel Electrophoresis (SDS-PAGE)

LcH-bound glycoconjugate preparations were isolated from the cell membranes of uncultured malignant cells from two patients with CLL and one patient with non-Hodgkin's lymphoma as described above for the antigen preparation (See above Section A. Leukemia Antigen Preparation).

The isolated glycoconjugates by this procedure are mostly glycoproteins. The three isolated glycoprotein preparations derived from three different patients were separately radiolabeled with ^{125}I using an IODO-GEN-coated Minisorp tube. To reduce the background radioactivity during the radioimmunoprecipitation, the three radiolabeled preparations were pretreated by incubating for 1 hour at 0° C with Pansorbin (Calbiochem), which had been coated with affinity-purified rabbit anti-mouse IgG antibodies and rabbit anti-LCH antibodies. For the specific immuno-precipitation, the pretreated radiolabeled samples were incubated, in duplicate, for 1 hour at 0° C with Pansorbin coated with affinity-purified rabbit anti-mouse IgG antibodies (RαMIgG) and mAb SN7 (IgG1). Control immunoprecipitates were prepared by using Pansorbin coated with RdMIgG and control mAb (anti-HLA DR) or control mouse IgGI (MOPC 195 variant). The specific and control immunoprecipitates were washed twice with Tris-HCl buffer (pH 7.2) containing 0.5% taurocholate (a detergent), 0.15 M NaCl, 2 mM EDTA, 0.1% bovine serum albumin, Trasylol (100 kallikrein units/ml), and 0.05% NaN_3 (Tris/taurocholate buffer). The immunoprecipitates were further washed twice with Tris/Renex 30 buffer (Tris buffer containing 0.5% Renex 30, a nonionic detergent, instead of 0.5% taurocholate) and once with 0.0625 M Tris-HCl buffer (pH 6.8) containing 0.01% cytochrome c. The

radiolabeled antigens of the washed immunoprecipitates were released from the Pansorbin by boiling for 3 min in the presence of 2.5% SDS and in the presence or absence of 0.1 M dithiothreitol. The released antigens were analyzed by SDS-PAGE using standard procedures, and autoradiograph was prepared by using a Kodak X-OMAT AR film and X-Omatic intensifying screen.

Preparation and Testing of Immunoconjugates Containing SN7

A. Preparation of Immunoconjugates

The purified SN7 antibody was covalently conjugated with the A chain of ricin, a plant toxin derived from the castor bean. The conjugation was carried out by a procedure which is based on a modification of our previously reported procedure [Cancer Research 48: 4673-4680 (1988)]. Briefly, the purified SN7 antibody in phosphate-buffered saline (PBS, pH 7.4) was treated with a 20-fold molar excess of SPDP, a heterobifunctional crosslinker, for 30 min at room temperature to introduce 2-pyridyl disulfide groups into the antibody molecule. The modified and dialyzed antibody was then mixed with a three-fold molar excess of the purified, freshly reduced ricin A-chain (RA) in PBS containing 1 mM EDTA and incubated at 4° C for 24 hr and at room temperature for 24 hr. The antibody-RA conjugates were separated from the unbound RA by gel filtration on a calibrated Sephacryl S-300 column. The remaining unconjugated antibody was removed from the conjugate fraction by chromatography on a Blue Sepharose column [Analytical Bichemistry 160: 440-443 (1987)].

B. Selective Killing of Human Leukemia-Lymphoma (HLL) Cells by Immunoconjugates

A direct test of in vitro cytotoxicity against target HLL cells and control cells was carried out as described previously [Cancer Research 44: 259-264 (1984)]. Briefly, cells were suspended in RPMI1640 medium supplemented with 5% fetal bovine serum, penicillin units/ml), streptomycin (100 µg/ml) and gentamicin (50 µg/ml) to a cell concentration of 7.5×10^5 cells/ml. One-ml portions of the cell suspensions were placed in individual wells (approximately 3.5 ml capacity) of Libro tissue culture plates. Immunoconjugates or PBS (control) was added, in triplicate, to the individual well cultures and the plates were placed in a humidified CO_2 (5%) incubator at 37° C. On days 2 and 3, a portion of each cell culture supernatant was replaced with fresh cell culture medium. A portion of each cell suspension was removed daily to determine viable cells using trypan blue.

RESULTS

Initial Characterization of Monoclonal Antibody

Reactivity of culture supernatants of hybridoma primary cultures and hybridoma clones derived from the primary cultures were initially characterized using a cellular radioimmunoassay (RIA) with various cultured and uncultured cells. The reactivity of SN7 with various malignant human hematopoietic cell lines is summarized in Table 1.

TABLE 1

Reactivity of SN7 with malignant human hematopoietic cell lines

The reactivity was determined using 20 µl of a 5-fold dilution of culture fluid of hybridoma T6-1G9 and 2 X 10^5 cells in each test by means of a cellular radioimmunoassay.

Cell line	Origin of cell line	Degree of Reactivity
B cell		
BALL-1	ALL	++++
BALM-2	ALL	++++
BALM-3	lymphocytic lymphoma	++
BALM-5	lymphocytic lymphoma	++
SU-DHL-4	histiocytic lymphoma	++++
Daudi	Burkitt's lymphoma	++++
Raji	Burkitt's lymphoma	++++
Ramos	Burkitt's lymphoma	+++
Pre-B cell		
NALM-1	CML-BC[a]	++
NALM-6	ALL	++
Non-T/Non-B cell		
KM-3	ALL	++++
NALM-16	ALL	+
T cell		
MOLT-4	ALL	-
JM	ALL	-
CCRF-HSB-2	ALL	-
Ichikawa	ALL	-
HPB-MLT	LTL[b]	-
HUT 78	Sezary syndrome	++++
Myeolo/monocytic cell		
ML-2	acute myelocytic leukemia	+
HL-60	acute promyelocytic leukemia	-
U937	histiocytic lymphoma	++
Myeloerythroid cell		
K562	CML-BC	-

TABLE 1(cont'd.)

Reactivity of SN7 with malignant human hematopoietic cell lines

Plasma cell		
ARH-77	Multiple myeloma	++++
HS	Multiple myeloma	++++
RPMI 8226	Multiple myeloma	+

[a] Chronic myelocytic leukemia in blast crisis
[b] Leukemic phase of T cell lymphoma

SN7 strongly reacted with all B cell type HLL cell lines tested; these cell lines were derived from patients with ALL, lymphocytic lymphoma, histiocytic lymphoma and Burkitt's lymphoma. SN7 also reacted with 2 of the 2 pre-B HLL cell lines tested, 2 of the 2 non-T/non-B (B-lineage) HLL cell lines tested, a T Sezary syndrome cell line and 2 of the 3 myelo/monocytic HLL cell lines tested. In addition, SN7 reacted with 3 of the 3 plasma cell lines derived from patients with multiple myeloma. However, SN7 did not react with 5 of the 6 T HLL cell lines tested, an immature promyelocytic leukemia cell line HL-60 nor with a myeloerythroid leukemia cell line K562. Further reactivity of SN7 was observed with 3 EB virus-transformed B cell lines CCRF-SB, RPMI 1788 and RPMI 8057.

SN7 showed minor or no significant reactivity against 7 normal (or near normal) bone marrow cell specimens derived from 7 different HLL patients in remission. The reactivity of SN7 with normal (or near normal) bone marrow cell specimens was further studied by FACS analysis and the results of FACS analysis were consistent with those of cellular RIA (see below). SN7 showed varying degrees of weak reactivity with 9 mononuclear cell specimens obtained from peripheral blood from 9 healthy donors. The reactivity of SN7 was further determined with different cell fractions obtained from normal peripheral blood. No significant reactivity of SN7 was observed with any T cell, granulocyte, erythrocyte or platelet fractions. However, varying degrees of a weak reaction of SN7 were observed with B cell and monocyte fractions. The degree of reactivity varied depending on the donor of the peripheral blood.

Reactivity with Uncultured HLL Cells

The reactivity of SN7 with fresh (uncultured) cell specimens derived from 76 patients with various HLL was determined by a cellular RIA and the results are summarized in Table 2.

Table 2

Reactivity of SN7 with uncultured human leukemia and lymphoma cells

Individual cell specimens were derived from peripheral blood, bone marrow or lymph node of different patients. In the cellular radioimmunoassay, three different controls were included with each cell specimen. One of these was control mouse IgG1 (10 µg/ml) in the hybridoma culture medium in place of the culture fluid of hybridoma. The other two controls were BALL-1 cells (a positive control) and MOLT-4 cells (a negative control) in place of the target cell specimen.

Disease of Patient	Reactivity[a]
B chronic lymphocytic leukemia	23/23
B prolymphocytic leukemia	6/6
Hairy cell leukemia	4/6
Non-T/non-B ALL[b]	7/10
B ALL	1/1
T ALL	0/5
Acute myelocytic leukemia	4/6
Acute myelomonocytic leukemia	2/3
Acute monocytic leukemia	1/2
Chronic myelocytic leukemia	2/3
Non-Hodgkin's lymphoma	11/11

a Number of reactive specimens per total number of specimens tested

b Pre-B ALL is included in this group

SN7 reacted with all 23 B chronic lymphocytic leukemia (CLL), all 6 B prolymphocytic leukemia, and all 11 non-Hodgkin's lymphoma specimens tested. Furthermore, SN7 reacted with more than 50% of the specimens tested for the following HLL; Hairy cell leukemia, non-T/non-B (including pre-B) ALL, acute myelocytic leukemia, acute myelomonocytic leukemia and chronic myelocytic leukemia. However, SN7 did not react with any of the 5 T ALL specimens tested. The reactivity of SN7 with uncultured HLL specimens is generally consistent with that of cultured cell lines (see Table 1).

FACS Analysis

Reactivity of SN7 with selected human cell specimens was investigated by FACS analysis. The results of FACS analysis were generally consistent with those of cellular IRA. The FACS analysis results are shown in Fig. 1 wherein target cells were allowed to react with SN7 or an isotype-matching control mouse IgG (MOPC 195 variant; IgG1) and stained with fluorescence-conjugated $F(ab')_2$ of sheep anti-mouse Ig antibodies. FACS analysis was carried out using FACS 440 (Becton Dickinson). The bone marrow specimens were obtained from two different ALL patients in remission and mononuclear cells were isolated for use in the test. The B CLL specimens shown in panels C and D were from two different CLL patients. BALL-1 (a B ALL cell line) and MOLT-4 (a T ALL cell line) were used as a positive and a negative control, respectively.

Of the two normal (or near normal) bone marrow specimens derived from ALL patients in remission (panels A and B in Fig. 1), a small population (less than 10%) of mononuclear cells of one bone marrow

specimen (No. 1) showed a w ak but significant reaction with SN7, whereas the other bone marr w specimen (No. 2) did not show any significant reaction with SN7. For two additional normal (not involved) bone marrow specimens taken from two patients with non-Hodgkin's lymphoma, a small population (approximately 4 and 7%, respectively) of mononuclear cells showed a w ak reaction with SN7. It is likely that some of the SN7 reactive cells in the above bone marrow specimens are residual malignant cells. In contrast to normal (or near normal) bone marrow cells, the majority (81 and 82%, respectively) of two B CLL cell specimens shown in Fig. 1 (panels C and D) strongly reacted with SN7. SN7 reacted strongly with virtually 100% (99%) of BALL-1 (panel E), but did not show any significant reactivity with MOLT-4 (panel F).

These FACS analysis results of normal bone marrow cells, CLL cells and cultured cell lines agreed well with the cellular RIA results of these same cell specimens (see above).

Radioimmunoprecipitation and SDS-PAGE of SN7 Antigen

In a separate experiment where detergent extracts of cell membranes of B CLL cells were fractionated, SN7 antigen was found to bind to an LcH column. In this experiment, therefore, HLL cell-membrane glycoprotein mixtures eluted from an LCH column were used for immunoprecipitation after radiolabeling. Three samples isolated from HLL cells derived from 3 HLL patients were tested; two of them were CLL patients and the other one was a non-Hodgkin's lymphoma patient. The immunoprecipitates obtained by using the radiolabeled samples and SN7 or an isotype-matching control mouse IgG (or control mAb) were analyzed by SDS-PAGE and autoradiographs were prepared. These results are shown in Fig. 2 wherein immunoprecipitates from ^{125}I-labeled cell membrane glycoprotein mixtures were obtained from CLL patient a (left panel), CLL patient b (middle panel) and a non-Hodgkin's lymphoma patient (right panel). The immunoprecipitation procedure used a 10-fold dilution of SN7 ascites (lanes A and D of left panel, and lanes B and E of right panel), purified SN7 antibody (lanes B and E of left panel, and lane B of middle panel), anti-HLA-DR mAb (Becton Dickinson; lane A of middle panel, and lanes A and D of right panel) and control mouse IgG (MOPC 195 variant; lanes C and F of left panel, lane C of middle panel, and lanes C and F of right panel). The immunoprecipitates were unreduced (lanes A, B and C of left and right panels) or reduced with dithiothreitol (lanes D, E and F of left and right panels and lanes A, B and C of middle panel) and analyzed by using 10% gels (left and middle panels) or 12% gels (right panel). The marker proteins (shown in K daltons) were ovalbumin (42.7), carbonic anhydrase (31.0), soybean trypsin inhibitor (21.5) and lysozyme (14.4).

The results of CLL sample a, CLL sample b and a non-Hodgkin's lymphoma sample are shown in the left, middle and right panels, respectively, of Fig. 2. Under unreduced conditions, both SN7 ascites (lane A of left panel and lane B of right panel) and purified SN7 antibody (lane B of left panel) immunoprecipitated a single major radiolabeled component of approximately 20,000 daltons. Under identical conditions, an isotype-matching control mouse IgG (MOPC 195 variant; IgG1) precipitated no significant component (lanes C of left and right panels) whereas anti-HLA-DR mAb (a control mAb obtained from Becton Dickinson) immunoprecipitated α (34,000 daltons) and β (28,000 daltons) subunits of HLA-DR antigens (lane A of right panel). Under reduced conditions, both SN7 ascites (lane D of left panel and lane E of right panel) and purified SN7 antibody (lane E of left panel and lane B of middle panel) again immunoprecipitated a single major component of 20,000 daltons. Under the same reduced conditions, the control mouse IgG precipitated no significant component (lanes F of left and right panels and lane C of middle panel) whereas anti-HLA-DR mAb immunoprecipitated α and β subunits of HLA-DR antigens (lane A of middle panel and lane D of right panel). These results show that SN7 antigen consists of a single polypeptide chain with an approximate molecular weight of 20,000. No significant difference was observed among SN7 antigens from three HLL samples (two CLL samples and a non-Hodgkin's lymphoma sample). In view of the 20,000 molecular weight and the fact that SN7 antigen was immunoprecipitated from the glycoprotein mixtures isolated by LcH affinity chromatography, SN7 antigen was designated as GP20.

Specific Cytotoxic Activity of Immunoconjugates Containing SN7

Immunoconjugates were prepared by covalently conjugating purified SN7 mAb to the A-chain subunit of ricin, a plant toxin. Ricin is composed of two disulfide-linked subunits, i.e., A and B chains. The B chain is a lectin which binds to galactose present n th surface of a wide variety of cells. The A chain is an enzyme which catalytically and irreversibly inhibits protein synthesis in the cytoplasm of the cells by acting on ribosomal RNA.

Ricin A-chain (RA) per se is not an effective cytotoxic agent against intact target cells because of its inability to bind efficiently to cell surfaces and to traverse the cell membranes. However, RA becomes effectively cytotoxic when delivered to the cytoplasm of the target cells by an appropriate delivery vehicle such as th ricin B-chain and an appropriate antibody. However, delivery of RA by ricin B-chain to target cells leads to the non-sel ctiv killing of virtually any mammalian cells because ricin B-chain binds to virtually all mammalian cells.

The cytotoxic activity of SN7-RA conjugates against two GP20-expressing HLL cell lines, BALL-1 and NALM-6 (See Table 1), and a GP20-non-expressing control cell line Ichikawa is summarized in Table 3.

Table 3

Specific cytotoxic activity of immunoconjugates prepared by conjugating purified mAb SN7 to ricin A-chain (RA)

GP20 (SN7 antigen)-expressing BALL-1 and NALM-6 cells and GP20-non-expressing control Ichikawa cells were separately cultured in individual wells of a tissue culture plate, in triplicate, in the absence (control) or in the presence of SN7-RA conjugates (2 X 10^{-8}M). The values given are the mean of triplicates ± the standard deviation.

		Viable cell number X10^{-4}				
Cell line	SN7-RA	Start	1 day	2 days	3 days	4 days
BALL-1	0	75±0	105±9	200±11	339±21	618±41
	2X10^{-8}M	75±0	1.4±0.6	0 ± 0	0 ± 0	0 ± 0
NALM-6	0	75±0	106±6	192±12	287±11	514±38
	2X10^{-8}M	75±0	25±4	0.9±0.2	0 ± 0	0 ± 0
Ichikawa	0	75±0	120±7	198±10	291±21	483±26
	2X10^{-8}M	75±0	156±24	271±15	364±21	472±21

After culturing for 2 days in the presence of SN7-RA conjugates, 100% and 99.5%, respectively, of BALL -1 and NALM-6 cells were killed whereas no significant killing was observed for control Ichikawa cells. After culturing for 3 and 4 days in the presence of SN7-RA conjugates, both BALL-1 and NALM-6 cells were completely killed whereas no significant killing was observed for Ichikawa cells (see Table 3). These results clearly show that SN7-RA conjugates selectively kill HLL cells which express GP20, an antigen defined by SN7. Furthermore, the cytotoxic activity of SN7-RA conjugates is extremely potent compared to cytotoxic activity of RA conjugates of other mAbs, e.g., Cancer Research 42: 457-464 (1982) and Cancer Research 48: 4673-4680 (1988). RA conjugates of mAbs generally need the presence of an appropriate potentiator to effectively kill target cells e.g., Cancer Research 48: 4673-4680 (1988).

It is important to note that SN7-RA conjugates strongly kill tumor cells in the absence of any potentiators such as NH_4Cl and monensin.

One promising approach for the treatment of HLL is bone marrow transplantation of patients who have received aggressive high dose chemotherapy and radiotherapy. The applicability of this approach, however, has been limited because the majority of the patients do not have suitable donors of normal bone marrow. One means of overcoming this limitation is to use autologous bone marrow transplantation, provided the bone marrow from the cancer patient can be made tumor cell-free in vitro. In this regard, since the anti-HLL mAb, SN7, generated according to the process of the present invention, shows selective binding to HLL cells in vitro, but does not react or shows only minor reactivity with normal bone marrow cells, it is believed that monoclonal antibody SN7 or SN7 conjugated with one or more of the various compounds and cytotoxic agents recited earlier herein will be extremely useful for the in vitro eradication of tumor cells from the bone marrow of HLL patients.

An appropriate method of treatment for carrying out the in vitro eradication of tumor cells in leukemia/lymphoma patients using mAb SN7 or a reactive fragment of SN7 would consist of removing bone marrow aspirates from the patient to be treated containing the leukemia and/or lymphoma cells, contacting in vitro the bone marrow aspirates with the mAb or a reactive fragment of same to eradicate the leukemia and lymphoma cells, thereby rendering the aspirates essentially free of leukemia and lymphoma cells, and

reintroducing the treated aspirates into the patient using known bone marrow transplantation techniques.

Another important therapeutic application of anti-HLL mAbs is their use in serotherapy. Over the past several years, a number of investigators have utilized murine mAbs for serotherapy of HLL patients by infusing r therwise introducing a cytotoxic amount of these mAbs into such patients, as for example, through the vascular fluid and/or directly into tumor sites. Although these therapeutic attempts were, in general, not successful in inducing complete remission of the treated patients, they provided information important for designing future serotherapy protocols. Particularly, it is believed that the use of immunoconjugates rather than unconjugated naked mAbs may prove effective for overcoming many of the problems presently associated with serotherapy. As mentioned above in connection with bone marrow transplantation, cytotoxic agents, drugs, toxins, radioisotopes, liposomes and many other agents may be attached directly or indirectly to appropriate anti-HLL mAbs in preparing such immunoconjugates for serotherapy.

In addition to using the above immunoconjugates, a new type of hybrid antibody which has been genetically engineered or reshaped, called "chimeric antibodies", may also be coupled with or incorporate mAb SN7 or an active fragment thereof for use in serotherapeutic procedures. Chimeric antibodies which combine rodent mAb variable regions with human antibody constant regions have two primary advantages over the conventional animal antibodies. First, the effector functions can be selected as desired, and second, the use of human rather than animal isotypes are reported to minimize the anti-globulin responses during therapy by avoiding anti-isotypic antibodies. This technology involves incorporating the rodent antigen binding site into human antibodies by transplanting the entire variable domain or only the antigen binding site from a rodent antibody. The production of chimeric antibodies has been reported in Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984), Nature 332:323-327 (1988) and BioTechniques, Volume 4 No. 3:214-220 (1986). Accordingly, it is further contemplated as fully within the scope of the present invention that a genetically engineered chimeric antibody incorporating the mAb disclosed herein, in whole or in part, may be successfully utilized for the treatment of a wide variety of human leukemias and lymphomas.

Still another potentially important therapeutic application of anti-HLL mAbs will be in preparing "internal image" anti-idiotype (Id) antibodies (termed Ab2β) and using Ab2β for prevention-therapy of HLL. Ab2β mimicking the original tumor associated antigen may be useful as an anti-tumor vaccine for inducing protective immunity against HLL. An idiotype or idiotypic determinant is an antigenic portion of an antibody that encompasses the variable region of the molecule. Within the variable region is the site where the antigen specifically binds to the antibody. The idiotype is often defined by an anti-idiotypic antibody (anti-Id), whereby the idiotype behaves as an antigen and induces the production of antibodies against itself.

The mechanism used to explain how an internal image anti-Id might mimic a tumor antigen and represent a vaccine is as follows. A host produces an antibody response (Ab1) against the tumor. An anti-Ab1 (or Ab2) response can be induced by immunizing appropriate animals (i.e., mice, rats, rabbits, goats, sheep, cows, horses, etc.) with Ab1. Some Ab2 which mimic the structure of the original tumor antigen may be referred to as an internal image anti-Id (Ab2β) and may be used as a substitute for the original tumor antigen. The Ab2β can be used to immunize a suitable host animal to produce an Ab3. The Ab3 can mimic the Ab1 and bind the tumor antigen. In certain instances where multi-determinant antigens are required for the induction of protective immunity, a pool of several Ab2β may be used as the vaccine. Recently, this inventor prepared Ab2β mimicking T HLL associated antigen, termed GP37, defined by mAb SN2 as reported in J. Immunol. 141:1398-1403 (1988] and J. Immunol. 139:1354-1360 (1987), the disclosures of which are hereby incorporated by reference. This inventor also generated Ab3 mimicking mAb SN2 (Ab1) [J. Immunol. 141:1398-1403 (1988)].

Internal image anti-Id (Ab2β) antibodies may likewise be prepared by immunizing an appropriate host animal with mAb SN7 or fragments of SN7. The resulting Ab2β thus produced would mimic GP20 and may have potential for inducing protective immunity against HLL in humans. In addition, the Ab2β or a fragment of same prepared using SN7 or SN7 fragments may also be used to immunize a suitable host animal to produce Ab3 antibodies mimicking the reactivity of SN7.

MAbs are also known to be highly useful for the diagnoses of various types of malignant tumors. The presence of leukemia or lymphoma cells in a patient may be determined by contacting an appropriate biological tissue cell specimen removed from the patient with a measured amount of mAbs or mAb reactive fragments and determining whether any reaction between the mAbs or fragments and the patient's cell specimen occurs using known techniques.

In particular, mAbs are curr ntly in use for localizing antigens. Tumor radioimaging procedures are well known in the art and involve conjugating the mAb or reactive fragment of same with a labeling amount of a known radioimaging compound and introducing the labeled conjugate thus formed into the vascular fluid of a host or patient. After a sufficient length of time to allow the labeled conjugate to react with the patient's leukemia/lymphoma target cells, the patient is subjected to known scintigraphic scanning procedures to

detect the location of the leukemia/lymphoma cells or tumor site(s).

MAb SN7 has been conjugated with radioactive ^{125}I and the resulting ^{125}I labeled SN7 was found to be stable. The SN7 conjugate maintained its antibody activity as demonstrated by RIA analysis.

Whole antibodies as well as reactive fragm nts including Fab, F(ab')$_2$ and Fv fragm nts may be conjugated to radioactive agents such as In-111 or I-123 to successfully identify and locate lymphoma lesions in cancer patients. Such conjugates may also be useful in therapy as radioactive agents are known to be cytotoxic to tumor cells. These techniques allow the discovery and treatment of unsuspected tumors such as those occurring in the lymph nodes.

Finally, the invention herein also contemplates providing mAb SN7 within a diagnostic kit for use in screening patients for the presence of lymphomas and leukemias. In general, such a screening procedure involves contacting an appropriate biological tissue cell specimen (i.e., blood, bone marrow, lymph node, etc.) removed from the test patient with a measured amount of mAb SN7 or a reactive fragment of SN7 and thereafter determining by known procedures whether a binding reaction between the mAb or fragment with the patient's specimen occurs. For purposes of viewing the reaction, suitable labeling agents or dyes are sometimes conjugated to the mAb or fragment. The conjugating reagent may also include one or more typical additives known in the art as, for example, buffering agents, agents for reducing background interference, and stabilizers.

When packaged as part of a diagnostic kit, suitable means may be provided within the kit for carrying out the diagnostic test. Such a kit may contain a means for obtaining the tissue cell specimen such as a syringe; the conjugated or unconjugated mAb or fragment and/or a labeling agent and ancillary agents such as slides, test tubes, etc.

The foregoing disclosure and the data presented herein demonstrate that the new mAb produced according to this invention, designated SN7, shows selective reactivity for a variety of human leukemia-lymphoma cells and defines a unique leukemia antigen designated as GP20. Test results using mAb SN7 against a wide variety of malignant and normal cell specimens suggests the usefulness of SN7 in the diagnosis and treatment of cancer patients suffering from a wide variety of leukemia-lymphomas.

The cell line designated T6-1G9, was deposited on May 18, 1989 at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. and was assigned the ATCC accession number HB 10151.

Although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be apparent to those skilled in the art without deviating from the spirit and scope of this invention. It is therefore intended that the present invention be limited only by the scope of the appended claims.

Claims

1. A monoclonal antibody or monoclonal antibody fragment characterizd in that it reacts with one or more leukemia lymphoma cell specimens selected from the group consisting of human B chronic lymphocytic leukemia cells; B prolymphocytic leukemia cells; Hairy cell leukemia cells; non-T acute lymphoblastic leukemia cells; acute myelocytic leukemia cells; acute myelomonocytic leukemia cells; acute monocytic leukemia cells; chronic myelocytic leukemia cells and non-Hodgkin's lymphoma cells.

2. The monoclonal antibody or fragment of Claim 1 which is directly or indirectly attached or complexed with a compound selected from the group consisting of cytotoxic agents, drugs, toxins or fragments thereof, hormones, enzymes, liposomes, radioactive agents, dyes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

3. The monoclonal antibody or fragment of Claim 2 wherein the fragment is selected from the group consisting of Fab, F(ab')$_2$ or Fv fragments.

4. The monoclonal antibody or fragment of Claim 1 which is produced by a hybridoma cell line designated T6-1G9 generated by fusing mouse myeloma cells with spleen cells from a suitable animal immunized with a non-T leukemia antigen preparation isolated from cell membranes of human acute lymphoblastic leukemia cells.

5. The monoclonal antibody or fragment of Claim 4 wherein said antigen preparation comprises an antig n having a molecular weight of about 20,000, designated GP20.

6. The monoclonal antibody or fragment of Claim 1 which does not react with T acute lymphoblastic leuk mia cells.

7. The monoclonal antibody or fragm nt of Claim 1 which exhibits no significant reactivity with human

normal T cells, granulocytes, erythrocytes or platelets.

8. The monoclonal antibody or fragment of Claim 1 which is of IgG1-k subclass.

9. A method of preparing the monoclonal antibody or fragment of Claim 1 characterized in that it comprises culturing a hybridoma cell line designated T6-1G9 in a suitable medium and recovering th monoclonal antibody or fragm nt from th culture supernatant of said hybridoma cell line.

10. A method of preparing the monoclonal antibody or fragment of Claim 1 characterized in that it comprises injecting into an appropriate animal a hybridoma cell line designated T6-1G9 and recovering the antibody or fragment from the malignant ascites or serum of said animal.

11. A method for detecting the presence of leukemia or lymphoma cells in a patient characterized in that it comprises contacting an appropriate biological tissue specimen removed from said patient with a measured amount of the monoclonal antibody or fragment of Claim 1 and determining whether any reaction between said monoclonal antibodies or fragments and the patient's specimen occurs.

12. A method for treating leukemia/lymphoma patients using the monoclonal antibody or fragment of Claim 1 characterized in that it comprises the steps of:

a) removing bone marrow aspirates from the patient to be treated containing the leukemia and lymphoma cells separately or combined,

b) contacting in vitro the bone marrow aspirates with said monoclonal antibody or fragment to eradicate the leukemia and lymphoma cells thereby rendering the aspirates essentially free of leukemia and lymphoma cells, and

c) reintroducing said treated aspirates into said patient using known bone marrow transplantation techniques.

13. The method according to Claim 11 wherein the monoclonal antibody or fragment of step (b) is first conjugated with a compound selected from the group consisting of cytotoxic agents, drugs, toxins or fragments thereof, hormones, enzymes, liposomes, radioactive agents, dyes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

14. The method according to Claim 13 wherein the fragment of step b) is selected from the group consisting of Fab, $F(ab')_2$ or Fv fragments.

15. A method for treating a leukemia/lymphoma patient which comprises introducing a cytotoxic amount of the monoclonal antibody or fragment of Claim 1 into the vascular fluid and/or directly into the tumor site of said patient.

16. The method according to Claim 15 wherein the monoclonal antibody or fragment is first conjugated with a compound selected from the group consisting of cytotoxic agents, drugs, toxins or fragments thereof, hormones, enzymes, liposomes, radioactive agents, dyes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

17. The method according to Claim 16 wherein the fragment is selected from the group consisting of Fab, $F(ab')_2$ or Fv fragments.

18. A method for detecting the location of leukemia/lymphoma cells within a host characterized in that it comprises the steps of:

a) conjugating the monoclonal antibody or fragment of Claim 1 with a labeling amount of a known radioimaging compound;

b) introducing the labeled conjugate of step a into the vascular fluid of the host:

c) and, after a sufficient length of time to allow the labeled conjugate to react with the host's leukemia/lymphoma cells, subjecting the host to known scintigraphic scanning procedures to detect the location of said leukemia/lymphoma cells within said host.

19. A diagnostic kit incorporating the monoclonal antibody or reactive fragment of Claim 1.

20. A hybridoma cell line designated T6-1G9.

21. A monoclonal antibody produced by hybridoma cell line T6-1G9 and clones thereof.

22. A monoclonal antibody or fragment of said monoclonal antibody produced by hybridoma cell line T6-1G9 and clones thereof.

23. A monoclonal antibody designated SN7.

24. An antigen designated GP20 which reacts with monoclonal antibody SN7 or fragments thereof.

25. A hybridoma cell line having the identifying characteristics of the hybridoma cell line of Claim 20.

26. A monoclonal antibody having the identifying characteristics of the monoclonal antibody of Claim 23.

27. The monoclonal antibody or fragment of Claim 23 which is directly or indirectly attached or complexed with a compound selected from the group consisting of cytotoxic agents, toxins or fragments

thereof, hormones, liposomes, radioactive agents, dyes, enzymes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

28. The monoclonal antibody or fragment of Claim 27 wherein the fragment is selected from the group consisting of Fab, $F(ab')_2$ or Fv fragments.

29. An Ab2β internal image anti-idiotype antibody or fragment thereof prepared from the monoclonal antibody or fragment of Claim 1.

30. An Ab3 anti-idiotype antibody or fragment thereof prepared from the Ab2β antibody or fragment of Claim 29.

31. A genetically engineered chimeric antibody incorporating the monoclonal antibody or monoclonal antibody fragment of Claim 1.

32. A monoclonal antibody or fragment of said monoclonal antibody characterized in that it:

a) reacts with human B chronic lymphocytic leukemia cells; B prolymphocytic leukemia cells; Hairy cell leukemia cells; non-T acute lymphoblastic leukemia cells; acute myelocytic leukemia cells; acute myelomonocytic leukemia cells; acute monocytic leukemia cells; chronic myelocytic leukemia cells and non-Hodgkin's lymphoma cells,

b) does not react with T acute lymphoblastic leukemia cells, and

c) does not react significantly with human normal T cells, granulocytes, erythrocytes or platelets.

33. The monoclonal antibody or fragment of Claim 32 which is directly or indirectly attached or complexed with a compound selected from the group consisting of cytotoxic agents, drugs, toxins or fragments thereof, hormones, enzymes, liposomes, radioactive agents, dyes, photodynamic agents, antibodies or fragments thereof, anti-idiotype antibodies or fragments thereof, chimeric antibodies or fragments thereof, and other monoclonal antibodies or fragments thereof.

34. The monoclonal antibody or fragment of Claim 33 wherein the fragment is selected from the group consisting of Fab, $F(ab')_2$ or Fv fragments.

35. The monoclonal antibody or fragment of Claim 32 which is produced by a hybridoma cell line designated T6-1G9 generated by fusing mouse myeloma cells with spleen cells from a suitable animal immunized with a non-T leukemia antigen preparation isolated from cell membranes of human acute lymphoblastic leukemia cells.

36. The monoclonal antibody or fragment of Claim 35 wherein said antigen preparation comprises an antigen having a molecular weight of about 20,000, designated GP20.

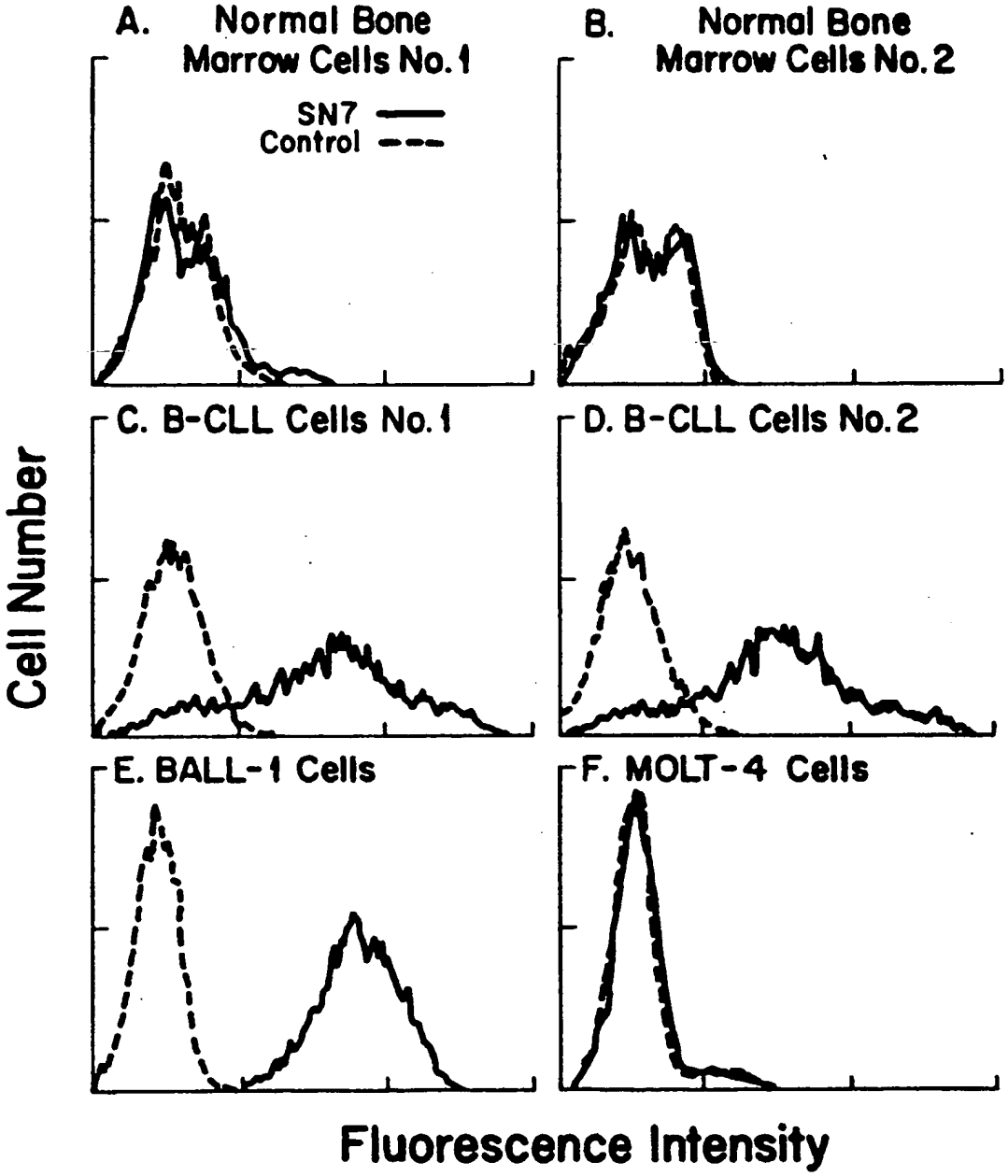
A. Normal Bone Marrow Cells No. 1
SN7
Control
B. Normal Bone Marrow Cells No. 2
C. B-CLL Cells No. 1
D. B-CLL Cells No. 2
E. BALL-1 Cells
F. MOLT-4 Cells
Cell Number
Fluorescence Intensity

FIG. 1

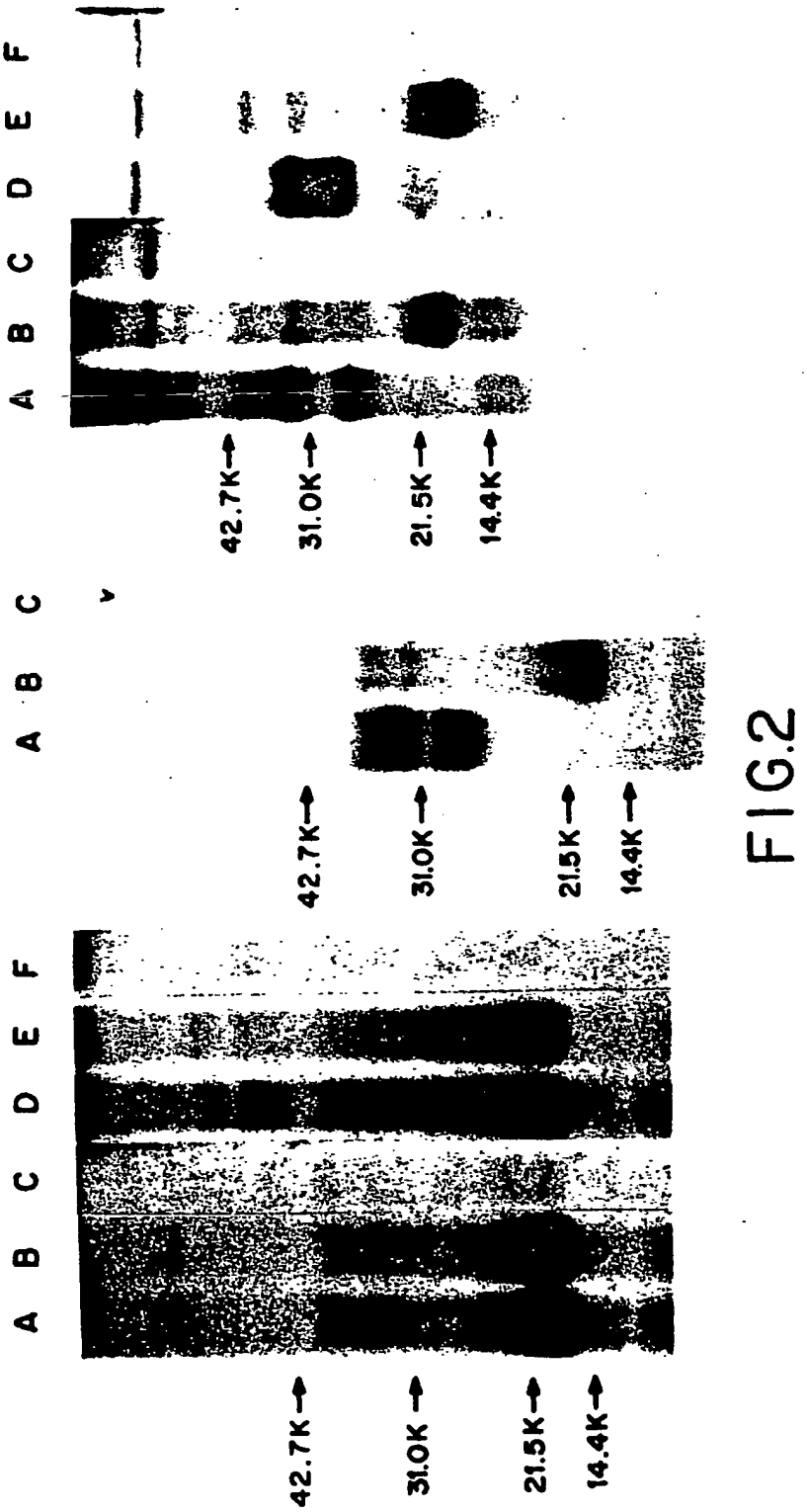
A B C D E F
42.7K
31.0K
21.5K
14.4K
A B C
42.7K
31.0K
21.5K
14.4K
A B C D E F
42.7K
31.0K
21.5K
14.4K

FIG.2

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number

EP 90 10 9768

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
	No further relavant document have been disclosed. ----		C 12 P 21/08 C 12 N 15/06 G 01 N 33/574 C 07 K 15/14

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 12 P

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1-11,19-36

Claims searched incompletely:

Claims not searched: 12-18

Reason for the limitation of the search:

Method for treatment of the human or animal body by surgery or therapy (see Art. 52(4) of the European Patent Convention).

Place of search	Date of completion of the search	Examiner
THE HAGUE	09-08-1990	NOOIJ

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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